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Diterpenoids and Bisnorditerpenoids from *Blumea aromatica*

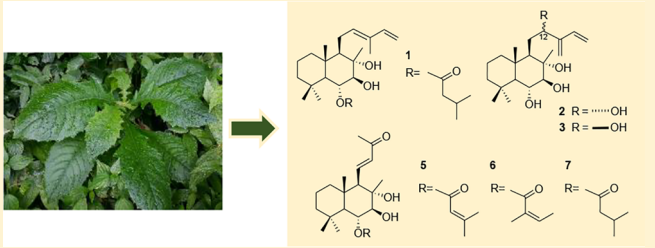
Chien-Chang Shen,[†] Wen-Chi Wei,[†] and Lie-Chwen Lin*[,†,‡]

[†]National Research Institute of Chinese Medicine, Ministry of Health and Welfare, Taipei, Taiwan

[‡]Graduate Institute of Integrated Medicine, China Medical University, Taichung, Taiwan

Supporting Information

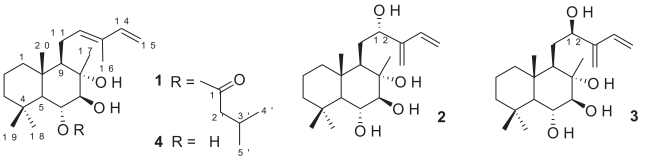
ABSTRACT: Three new labdane-type diterpenoids, 6α-*O*-isovalerylnidorellol (**1**), (12*S*)-blumdane (**2**), and (12*R*)-epiblumdane (**3**), and three new bisnorditerpenoids, 6α-*O*-(3-methyl-2-butenoyl)sterebin A (**5**), 6α-*O*-angeloylsterebin A (**6**), and 6α-*O*-isovalerylsterebin A (**7**), plus 17 known compounds were isolated from *Blumea aromatica*. Their structures of the new compounds were proposed by detailed spectroscopic analysis. The absolute configuration at C-12 of blumdane (**2**) was determined by the modified Mosher's method. The anti-inflammatory and anti-immunosuppressive effects of these isolated compounds were assessed. Compounds **9**, **16**, and **23** (at 40 μM) showed a slight suppression of TNF-α production, but no or little effect on the expression of PD-L1 in granulocytic myeloid-derived suppressor cells was observed for all test compounds.

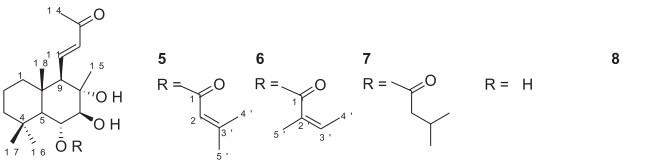


Blumea aromatica DC. (Asteraceae) is a tall perennial herb occurring in forests at low to medium elevations in Taiwan.[1] The whole herbs of *B. aromatica* have been used as an herbal remedy in folk medicine to treat leukemia, arthritis, headache, and skin eczema.[2] A previous chemical study on this species reported the identification of a xanthoxylin, an anthraquinone, and flavonoids.[3]

TNF-α, a key proinflammatory cytokine, plays an important role in the acute immune response and systemic inflammation.[4,5] Myeloid-derived suppressor cells (MDSCs) have been demonstrated to induce immune suppression, resulting in protecting tumor cells from immune surveillance. Programmed cell death protein 1 (PD-L1), an immune checkpoint molecule, plays a key role in cancer immune evasion.[6,7] In order to develop new bioactive components from natural resources, a systemic chemical study of *B. aromatica* was carried out, along with an anti-inflammatory assay on TNF-α expression in lipopolysaccharide (LPS)-stimulated RAW 264.7 murine macrophages and an anti-immunosuppressive assay on PD-L1 expression in LPS-activated MDSCs. Herein are reported the isolation and structural determination of six new compounds, **1**−**3** and **5**−**7**, together with the evaluation of the anti-inflammatory and anti-immunosuppressive activities for these compounds and several known constituents in *B. aromatica*.

The *B. aromatica* EtOH extract was fractionated by solvent partition. Each fraction was subjected subsequently to a coalescence of chromatographic steps, as described in the Experimental Section, to afford compounds **1**−**23**. Among these compounds, **1**−**3** are new labdane-type diterpenoids and **5**−**7** are new bisnorditerpenoids. The other 17 compounds were known previously and were determined from their ^{1}H and ^{13}C NMR and MS data as 6α-hydroxynidorellol (**4**),[8]





sterebin A (**8**),[9] 4,5-di-*O*-caffeoylquinic acid (**9**),[10] 4,5-di-*O*-caffeoylepiquinic acid (**10**),[10] 3,5-di-*O*-caffeoylquinic acid (**11**),[10] 1,5-di-*O*-caffeoylquinic acid (**12**), butyl 4,5-di-*O*-caffeoylquinate (**13**),[10] methyl 4,5-di-*O*-caffeoylquinate (**14**),[10] 5-*O*-caffeoylquinic acid (**15**),[8] 3,6,4′-trimethoxy-5,7-dihydroxyflavone (**16**), kaempferol-3-*O*-glucoside (**17**),[11] fumaric acid (**18**), 2-*sec*-butylmaleic acid (**19**), 2-*sec*-butylmaleic acid 4-methyl ether (**20**), β-sitosterol (**21**), indole-3-carboxaldehyde (**22**),[12] and (*Z*)-2-(2,4-dihydroxy-2,6,6-trimethylcyclohexylidene)acetic acid (**23**).[13]

6α-*O*-Isovalerylnidorellol (**1**), $[\alpha]_D$ +56.6 (*c* 0.1, MeOH), gave signals for 25 carbons in its ^{13}C NMR spectrum, including seven methyl carbons, five secondary sp^3 carbons, five tertiary sp^3 carbons, three quaternary sp^3 carbons, one secondary sp^2 carbon, two tertiary sp^2 carbons, one quaternary sp^2 carbon, and one carbonyl carbon. Two tertiary and one quaternary sp^3

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Table 1. ^{1}H NMR Spectroscopic Data for Compounds 1−4 (δ in ppm and J in Hz)[a]

position	1	2	3	4
1	0.92[b]/1.53, d (14.0)	1.05, m/1.64, m	1.05[b]/1.53, m	0.89, dt (13.5, 4.0) /1.49, d (13.5)
2	1.38[b]/1.60, dt (13.5, 3.5)	1.44, m/1.64, m	1.41, dt (13.8, 4.2)/1.63, d (13.8)	1.33[b]/1.58, dt (13.0, 3.5)
3	1.22, td (14.0, 4.0)/1.31[b]	1.24, td (13.6, 4.0)/1.35, d (13.6)	1.25[b]/1.31[b]	1.21, td (14.5, 4.0)/1.32[b]
5	1.39, d (11.5)	1.15[b]	1.13[b]	1.12[b]
6	5.17, dd (11.5, 9.5)	3.54, dd (10.8, 9.6)	3.54, dd (10.8, 9.6)	3.55, dd (11.0, 9.0)
7	3.38, d (9.5)	3.30[b]	3.30[b]	3.27, d (9.5)
9	1.36, dd (7.0, 3.0)	1.50, t (3.6)	1.58[b]	1.33, m
11	2.21, dd (15.5, 7.5)	1.57, ddd (15.0, 10.2, 4.2)/1.72, ddd (15.0, 3.0, 2.4)	1.53[b]/1.88, m	2.18, dt (16.0, 8.0)
	2.42, br d (15.5)			2.39, dd (16.0, 4.0)
12	5.52, t (7.0)	4.22, br d (10.2)	4.57, dd (9.6, 3.6)	5.51, t (7.0)
14	6.32, dd (17.5, 11.0)	6.37, dd (17.4, 10.8)	6.38, dd (17.4, 10.8)	6.32, dd (17.5, 10.5)
15	4.83[b]/5.02, d (17.5)	5.07, d (10.8)/5.34, d (17.4)	5.09, d (10.8)/5.44, dd (18.0, 1.2)	4.83[b]/5.01, d (17.0)
16	1.74, s	5.11, s/5.28, s	5.24, s/5.25, s	1.73, s
17	1.15, s	1.14, s	1.12, s	1.11, s
18	0.98, s	1.17, s	1.17, s	1.15, s
19	0.89, s	1.00, s	1.00, s	1.00, s
20	0.98, s	0.85, s	0.85, s	0.93, s
2′	2.17[b]/2.33[b]			
3′	2.13, m			
4′	0.98, d (6.5)			
5′	0.99, d (7.5)			

[a]Compounds **1**−**4** were measured in methanol-d_4. [b]Signal partially obscured.

carbons were oxygenated. The UV absorption at 224 nm (log ε 3.44) and the IR absorption bands at 3447, 1722, and 1635 cm^{-1} suggested the occurrence of hydroxy and carbonyl groups and a diene system in the molecule. The ^{1}H NMR spectrum of **1** showed seven methyl groups [δ_H 0.89 (s, H_3-19), 0.98 (s, H_3-18/H_3-20), 0.98 (d, J = 6.5 Hz, H_3-4′), 0.99 (d, J = 7.5 Hz, H_3-5′), 1.15 (s, H_3-17), and 1.74 (s, H_3-16)], four olefinic protons [δ_H 4.83 (H-15a), 5.02 (d, J = 17.5 Hz, H-15b), 5.52 (t, J = 7.0 Hz, H-12), and 6.32 (dd, J = 17.5, 11.0 Hz, H-14)], and two oxymethine groups [δ_H 3.38 (d, J = 9.5 Hz, H-7) and 5.17 (dd, J = 11.5, 9.5 Hz, H-6)]. The COSY spectrum of **1** indicated five isolated spin systems as follows: H_2-1/H_2-2/H_2-3; H-5/H-6/H-7; H-9/H_2-11/H-12; H-14/H_2-15; H_3-4′,H_3-5′/H-3′/H_2-2′. Protons attached to carbons were verified by the HMQC spectrum, and the structure of **1** was elucidated by HMBC analysis. The NMR signals of **1** resembled those of 6α-hydroxynidorellol (**4**)[8] except for the low-field-shifted signal of H-6 and the signals for an isovaleryl group at δ_H 0.98 (d, J = 6.5 Hz, H_3-4′), 0.99 (d, J = 7.5 Hz, H_3-5′), 2.13 (m, H-3′), 2.17 (d, J = 7.5 Hz, H-2′), 2.33 (d, J = 9.5 Hz, H-2′), and δ_C 174.7 (C-1′) attributed to a carbonyl group, 45.1 (C-2′), 26.3 (C-3′), and 22.9 (C-4′ and C-5′). The molecular formula of $C_{25}H_{42}O_4$ for **1** was deduced by ^{13}C NMR and HRESIMS. In the HRESIMS, a [M − isovaleryl]$^+$ ion at m/z 321.2434 (calcd for $C_{20}H_{33}O_3$, 321.2424) was observed. HMBC correlations of C-1′ with H-6 and H-3′ confirmed the linkage of a 6-O-isovaleryl group. In the NOESY spectrum, correlations of H-6/H-17, H-19, H-20 and H-7/H-5 indicated the configurations of the 6α-isovaleryloxy, 7β-hydroxy, and 8β-methyl substituents, and correlations of H-12/H-14 and H-16/Ha-15 were consistent with an E-type diene configuration. Therefore, **1** was proposed as 6α-O-isovalerylnidorellol.

(12S)-Blumdane (**2**), $[\alpha]_D$ +28.0 (c 0.1, MeOH), was determined to have a molecular formula of $C_{20}H_{34}O_4$ based on the ^{13}C NMR data and the [M + Na]$^+$ ion peak at m/z 361.2359 (calcd for $C_{20}H_{34}O_4Na$, 361.2349) in the HRESIMS, one oxygen atom more than 6α-hydroxynidorellol (**4**).[8] The UV absorption at 223 nm (log ε 3.80) and the IR absorption bands at 3422 and 1653 cm^{-1} revealed the presence of a hydroxy group and a diene system in the molecule. Comparison of the ^{1}H and ^{13}C NMR spectra of **2** with those of **4** suggested that the main differences were from the side chain attached at C-9. The ^{1}H NMR spectrum of the side chain in **2** showed signals for an aliphatic methylene [δ_H 1.57, ddd, J = 15.0, 10.2, 4.2 Hz, H-11a), 1.72 (d, J = 15.0, 3.0, 2.4 Hz, H-11b)], an oxygenated methine (δ_H 4.22, br d, J = 10.2 Hz, H-12), two sets of olefinic methylenes (δ_H 5.07/5.34, H_2-15; 5.11/5.28, H_2-16), and an olefinic methine (δ_H 6.37, dd, J = 17.4, 10.8 Hz, H-14). The corresponding proton-attached carbons exhibited signals at δ_C 34.2 (t, C-11), 73.6 (d, C-12), 137.8 (d, C-14), 114.3 (t, C-15), and 114.1 (t, C-16). These signals and the signal at δ_C 151.6 (s, C-13) were assigned as the 2-hydroxy-3-methylene-4-penten-1-yl moiety in **2**, which was confirmed by COSY and HMBC experiments. The absolute configuration of OH-12 was determined by the modified Mosher's method.[14,15] The (*S*)- and (*R*)-MTPA esters of **2** (**2-SE** and **2-RE**) were prepared, respectively, and the differences in the chemical shifts ($\Delta\delta = \delta_S - \delta_R$) of **2-SE** and **2-RE** were calculated. Positive $\Delta\delta$ values were observed for H-14, H-15, and H-16, whereas negative $\Delta\delta$ values was observed for H_3-17. Based on the above results, a 12S configuration was deduced and the structure of **2** was established as shown.

(12R)-Epiblumdane (**3**), $[\alpha]_D$ +47.2 (c 0.1, MeOH), showed a similar UV spectrum and gave an identical sodiated molecular ion [M + Na]$^+$ at m/z 361.2354 (calcd for $C_{20}H_{34}O_4Na$, 361.2349) in the HRESIMS as **2**. As seen in Tables 1 and 3, these two compounds exhibited similar 1D NMR data except that the signal at δ_H 4.57 (dd, J = 9.6, 3.6 Hz) for H-12 of **3** shifted to a lower field than the H-12 signal of **2**. Compared to the signals of **2**, the C-12 signal (δ_C 87.3, d) in **3** was observed at a low field and the C-11 (δ_C 34.2, t) and

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Table 2. ^{1}H NMR Spectroscopic Data for Compounds 5-8 (δ in ppm and J in Hz)[a]

position	5	6	7	8
1	1.09, t (7.0)/ 1.38, m	0.96[b]/ 1.39, m	0.92[b]/1.36, m	0.89, m/ 1.32[b]
2	1.40[b]/ 1.61, m	1.39, m/ 1.61, m	1.38, m/1.62, m	1.36[b]/ 1.61, m
3	1.32[b]/1.36[b]	1.27[b]/1.33[b]	1.25[b]/1.32[b]	1.22[b]/1.36[b]
5	1.42, d (11.5)	1.46, d (11.5)	1.41, d (11.0)	1.13, d (11.0)
6	5.26, dd (11.5, 10.0)	5.35, dd (11.5, 9.5)	5.26, dd (11.5, 9.5)	3.64, dd (10.5, 9.5)
7	3.43, d (9.5)	3.48, d (10.0)	3.41, d (10.0)	3.30[b]
9	2.04, d (10.5)	2.06, d (10.0)	2.03, d (10.0)	2.00, d (10.5)
11	6.93, dd (16.0, 10.5)	6.93, dd (16.0, 10.5)	6.91, dd (15.5, 10.5)	6.91, dd (15.5, 10.5)
12	6.14, d (16.0)	6.15, d (16.0)	6.14, d (15.5)	6.13, d (15.5)
14	2.28, s	2.28, s	2.28, s	2.27, s
15	1.26, s	1.27, s	1.25, s	1.21, s
16	0.98, s	0.97, s	1.01, s	1.17, s
17	0.92, s	0.95, s	0.92, s	1.03, s
18	1.16, s	1.17, s	1.14, s	1.08, s
2′	5.72, s		2.18, d (7.5)/ 2.33, d (9.0)	
3′		6.12, dq (7.0, 1.5)	2.14, m	
4′	2.19, s	2.00, d (7.0)	0.98, d (6.5)	
5′	1.91, s	1.91, s	0.99, d (6.5)	

[a]Compounds **5**–**8** were measured in methanol-d_4. [b]Signal partially obscured.

C-13 (δ_C 147.7, s) signals shifted to a higher field. From the above evidence, **3** was deduced to be an epimer of **2** with the opposite configuration at C-12, and the structure of **3**, (12*R*)-epiblumdane, was determined as shown.

6α-*O*-(3-Methyl-2-butenoyl)-sterebin A (**5**), $[\alpha]_D$ +42.0 (*c* 0.1, MeOH), was concluded to have a molecular formula of $C_{23}H_{36}O_5$, dependent on the ^{13}C NMR data and the $[M + Na]^+$ ion peak at *m*/*z* 415.2469 (calcd for $C_{23}H_{36}O_5Na$, 415.2455) in the HRESIMS. The UV absorption at 221 nm (1log ε 4.12) and the IR absorption bands at 3444, 1719, and 1648 cm^{-1} proposed the presence of hydroxy, carbonyl, and olefinic groups in the molecule. The ^{1}H NMR signals of **5** at δ_H 2.28 (3H, s, H_3-14), 6.14 (1H, d, J = 16.0 Hz, H-12), and 6.93 (1H, dd, J = 16.0, 10.5 Hz, H-11) and the ^{13}C NMR signals at δ_C 146.0 (d, C-11), 136.9 (d, C-12), 200.9 (s, C-13), and 27.1 (q, C-14) were assigned for a *trans*-α,β-unsaturated methyl ketone group. Further, the ^{1}H NMR spectrum contained signals for four tertiary methyls at δ_H 0.92 (H_3-17), 0.98 (H_3-16), 1.16 (H_3-18), and 1.26 (H_3-15) and two oxygenated methines at δ_H 5.26 (dd, J = 10.0, 11.5 Hz, H-6) and 3.43 (d, J = 9.5 Hz, H-7). The coupling constants of the latter two oxymethine signals supported the presence of 1,2-*trans*-diequatorial diol system in a cyclohexane ring for **5**. The COSY, HMQC, and HMBC spectra indicated that **5** is a derivative of sterebin A (**8**), a bisnorditerpene.[9] Moreover, the ^{1}H NMR signals at δ_H 5.72 (s, H-2′) and 1.91/2.19 (each s, H_3-5′, 4′) and the ^{13}C NMR signals at δ_C 168.0 (s, C-1′), 117.8 (d, C-2′), 158.5 (s, C-3′), and 20.4/27.5 (each q, C-4′, 5′) were assigned for a 3-methyl-2-butenoyl group. An HMBC experiment showed the correlation of H-6 to C-1′, suggesting the linkage of the 3-methyl-2-butenoyl group at C(6)-*O*–. NOESY correlations of H-6/H_3-15, H_3-17, H_3-18 and H-7/H-5, H-9 were consistent with the configurations of 6α-

Table 3. ^{13}C NMR Spectroscopic Data for Compounds 1–8 (δ in ppm)[a]

	1	2	3	4	5	6	7	8
position	δ_C, type	δ_C, type	δ_C, type	δ_C, type	δ_C, type	δ_C, type	δ_C, type	δ_C, type
1	41.3, CH_2	40.8, CH_2	41.1, CH_2	41.4, CH_2	42.2, CH_2	42.1, CH_2	42.1, CH_2	42.3, CH_2
2	19.0, CH_2	19.1, CH_2	19.1, CH_2	19.2, CH_2	19.0, CH_2	19.0, CH_2	19.0, CH_2	19.2, CH_2
3	44.8, CH_2	44.9, CH_2	45.0, CH_2	45.0, CH_2	44.7, CH_2	44.7, CH_2	44.6, CH_2	44.8, CH_2
4	34.3, C	34.9, C	34.8, C	34.7, C	34.5, C	34.5, C	34.4, C	34.9, C
5	57.9, CH	58.9, CH	58.8, CH	58.8, CH	57.9, CH	57.9, CH	57.6, CH	58.6, CH
6	74.4, CH	73.0, CH	73.0, CH	73.1, CH	73.2, CH	73.8, CH	74.1, CH	72.9, CH
7	84.1, CH	86.2, CH	86.1, CH	86.1, CH	83.8, CH	83.9, CH	83.8, CH	85.7, CH
8	77.7, C	76.6, C	77.7, C	77.5, C	76.5, C	76.6, C	76.5, C	76.3, C
9	61.1, CH	58.1, CH	56.3, CH	61.3, CH	64.8, CH	64.7, CH	64.8, CH	65.0, CH
10	40.4, C	40.2, C	40.2, C	40.1, C	39.3, C	39.3, C	39.3, C	39.0, C
11	24.8, CH_2	34.2, CH_2	29.2, CH_2	24.8, CH_2	146.0, CH	145.9, CH	145.9, CH	146.3, CH
12	137.8, CH	73.6, CH	87.3, CH	138.1, CH	136.9, CH	136.9, CH	136.9, CH	136.7, CH
13	133.0, C	151.6, C	147.7, C	132.9, C	200.9, C	200.8, C	200.8, C	200.9, C
14	143.0, CH	137.8, CH	137.7, CH	143.1, CH	27.1, CH_3	27.1, CH_3	27.1, CH_3	27.1, CH_3
15	110.1, CH_2	114.3, CH_2	115.0, CH_2	110.0, CH_2	19.3, CH_3	19.3, CH_3	19.3, CH_3	19.3, CH_3
16	12.0, CH_3	114.1, CH_2	116.4, CH_2	12.0, CH_3	36.6, CH_3	36.6, CH_3	36.6, CH_3	37.0, CH_3
17	19.2, CH_3	20.1, CH_3	19.7, CH_3	19.2, CH_3	22.5, CH_3	22.6, CH_3	22.5, CH_3	22.5, CH_3
18	36.7, CH_3	36.9, CH_3	37.0, CH_3	37.0, CH_3	17.7, CH_3	17.7, CH_3	17.6, CH_3	17.8, CH_3
19	22.5, CH_3	22.5, CH_3	22.4, CH_3	22.5, CH_3				
20	17.2, CH_3	17.4, CH_3	17.2, CH_3	17.4, CH_3				
1′	174.7, C				168.0, C	169.3, C	174.6, C	
2′	45.1, CH_2				117.8, CH	129.8, C	45.1, CH_2	
3′	26.3, CH				158.5, C	139.1, CH	26.4, CH	
4′	22.9, CH_3				20.4, CH_3	20.8, CH_3	22.9, CH_3	
5′	22.9, CH_3				27.5, CH_3	16.0, CH_3	22.9, CH_3	

[a]Compounds **1**–**8** were measured in methanol-d_4.

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substituted, 7β-hydroxy, and 8β-methyl substituents as being the same as those of sterebin A. Correspondingly, the structure of **5** was established as shown.

Compounds **6** and **7** exhibited similar ^{1}H and ^{13}C NMR spectra (Table 1) with signals stemming from a bisnorditerpene skeleton, corresponding to the framework of sterebin A (**8**).[9] HRESIMS analyses suggested that **6** and **7** have the molecular formulas $C_{23}H_{36}O_5$ and $C_{23}H_{38}O_5$, respectively. In the ^{1}H NMR spectra of **6** and **7**, the characteristic signals of sterebin A were observed, along with an angeloyl group [δ_H 6.12 (dq, H-3′), 2.00 (d, H_3-4′), and 1.91 (s, H_3-5′)] in **6** and an isovaleryl group [δ_H 2.17/2.33 (each d, H_2-2′), 2.14 (m, H-3′), and 0.98/0.99 (each d, H_3-4′, 5′)] in **7**, which were confirmed by COSY, NOESY, HMQC, and HMBC experiments. HMBC correlations from H-6 to C-1′ indicated the location of the angeloyl group in **6** and the isovaleryl group in **7** at C(6)-*O*−, respectively. Thus, the structures of **6** and **7** were determined as 6α-*O*-angeloylsterebin A and 6α-*O*-isovalerylsterebin A, respectively.

The isolated compounds were assessed for their inhibitory effects on TNF-α expression in LPS-stimulated RAW 264.7 murine macrophages. The ELISA results showed compounds **9**, **16**, and **23** at 40 μM slightly suppressed the production of TNF-α. The inhibition rates of compounds **9**, **16**, and **23** are 15.5 ± 0.7%, 24 ± 2.2%, and 27.5 ± 5.1%, respectively. The inhibitory effect of the isolated compounds on PD-L1 expression in LPS-activated MDSCs was also evaluated. The results showed that LPS strongly up-regulated PD-L1 expression, but the test compounds showed no or little effect on the expression of PD-L1 in granulocytic MDSCs (gMDSCs).

EXPERIMENTAL SECTION

General Experimental Procedures. The instruments for measuring optical rotations, recording UV, IR, NMR, ESIMS, and HRESIMS spectra, and HPLC used to isolate components are the same as reported previously.[16] Silica gel (Merck, 63−200 μm; Fuji Silysia, 40−70 μm) and Sephadex LH-20 were used for column and gel filtration chromatography, respectively. Precoated silica gel TLC plates (Merck, F254) were used for analytical or preparative thin-layer chromatography, and spots were visualized by UV light (254 nm) or by spraying with 20% H_2SO_4 in EtOH followed by heating. Cosmosil 5C_{18}-AR-II (10 × 250 mm; particle size: 5 μm; Nacalai Tesque, Kyoto, Japan) and LiChrospher 60 RP-select B (10 × 250 mm; particle size: 5 μm; Merck KGaA, Darmstadt, Germany) columns were used for semipreparative HPLC separation.

Plant Material. The whole plant of *B. aromatica* was collected in August 2017 in Hsinchu, Taiwan, and identified by Dr. Cheng-Jen Chou, retired Research Fellow, National Research Institute of Chinese Medicine, Taipei, Taiwan. A voucher specimen (NRICM-30-BA-01) is deposited in the Herbarium of National Research Institute of Chinese Medicine, Taipei, Taiwan.

Extraction and Isolation. The air-dried whole plant of *B. aromatica* (1.8 kg) was cut into segments, extracted three times with 30 L of EtOH at 50 °C, and concentrated to afford an EtOH extract (166.9 g). The EtOH extract was suspended in H_2O (0.5 L) and then extracted sequentially with *n*-hexane, EtOAc, and *n*-BuOH to give *n*-hexane, EtOAc, *n*-BuOH, and H_2O extracts, respectively. Due to similar TLC profiles of *n*-hexane and EtOAc extracts, these extracts were combined (as A-extract) for the following separation. A-extract (61 g) was separated by silica gel column chromatography (8.5 × 55 cm, 94.5 g) using a gradient eluent of EtOAc in *n*-hexane (10% → 100%) to afford 20 fractions (Fr. 1−20). Fr. 6 (1.159 g) was chromatographed by semipreparative HPLC (C_{18}, eluent: 70% CH_3CN/H_2O, flow rate: 4 mL/min) to give **1** (37 mg). Compound **21** (40 mg) was obtained from Fr. 7 and Fr. 8 after recrystallization from acetone. Fr. 12 (1.56 g) was chromatographed on a Sephadex LH-20 column (3 × 57 cm) eluting with EtOH to give **16** (93 mg). Fr. 15 (4.61 g) and Fr. 16 (2.34 g) were combined and then separated on a silica gel column (2.6 × 20 cm) using 40% EtOAc/*n*-hexane as the mobile phase to give subfractions 15A−15C. Fr. 15B (3.93 g) was subjected to separation over a Sephadex LH-20 column (4.5 × 50 cm), eluting with 5% EtOAc/MeOH, to give Fr. 15B2, which recrystallized from MeOH to afford **4** (340 mg). Fr.15C (1.06 g) was subjected to a Sephadex LH-20 column (3 × 54 cm) eluting with MeOH to give Fr. 15C1−15C4. Compound **22** (1.2 mg) was obtained from Fr. 15C3 by semipreparative HPLC (C_{18}, eluent: 30% CH_3CN/H_2O, flow rate: 4 mL/min). Fr. 15C2 (0.76 g) was chromatographed by semipreparative HPLC (C_{18}, elution program: 40%−60%−60%−90% CH_3CN/H_2O during 0−7−21−22 min, flow rate: 4 mL/min) to afford **4** (50 mg), **23** (22 mg), and Fr. 15C2m. Fr. 15C2m was further separated by preparative TLC (5% MeOH/$CHCl_3$) to afford **2** (12 mg) and **3** (15 mg). Fr. 17 (6.4 g) was subjected to silica gel column chromatography (2.6 × 20 cm), eluting with 80% EtOAc/*n*-hexane, to give subfractions 17A−17D. Fr. 17B (1.96 g) was separated on a Sephadex LH-20 (5% EtOAc/methanol) and semipreparative HPLC column (C_{18}, eluent: 65% $MeOH/H_2O$, flow rate: 4.5 mL/min) to afforded **5** (4.0 mg), **6** (30 mg), and **7** (35 mg). Fr. 17D (889 mg) was separated on a Sephadex LH-20 (MeOH) and semipreparative HPLC column (C_{18}, eluent: 50% CH_3CN/H_2O, flow rate: 4 mL/min) to give **8** (9.3 mg). The *n*-butanol extract (14.3 g) was separated by a Sephadex LH-20 column (7.2 × 65 cm) eluting with EtOH to give seven fractions (Fr. B1−B7). Compounds **18** (1.17 g) and **19** (204 mg) precipitated successively from Fr. B4 and were recrystallized further from MeOH. The filtrate of Fr. B4 was chromatographed by semipreparative HPLC (C_{18}, elution program: 25%−25%−90% CH_3CN/0.1% formic acid during 0−10−20 min, flow rate: 4 mL/min) to afford compounds **13** (15 mg), **17** (24 mg), and **20** (3.2 mg). Fr. B5 was subjected to semipreparative HPLC (RP-select B, eluent: 10% to 50% of CH_3CN/0.1% formic acid over 30 min, flow rate: 4 mL/min) to give **12** (2.4 mg) and **15** (80 mg). Fr. B6 (1.79 g) was chromatographed on a Sephadex LH-20 (MeOH) and semipreparative HPLC column (RP-select B, eluent: 20% CH_3CN/0.1% formic acid, flow rate: 4 mL/min) to afford compounds **9** (67 mg), **10** (20 mg), **11** (23 mg), and **14** (6.4 mg).

*6α-O-Isovalerylnidorellol (**1**):* $[\alpha]^{26}_D$ +56.6 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 224 (3.44) nm; IR (neat) ν_{max} 3447, 2960, 2930, 1722, 1635, 1467, 1393, 1371, 1193, 1005 cm^{-1}; ^{1}H NMR (CD_3OD) see Table 1; ^{13}C NMR (CD_3OD) see Table 3; HRESIMS *m/z* 321.2434 $[M - C_5H_9O]^+$ (calcd for $C_{20}H_{33}O_3$, 321.2424).

*(12S)-Blumdane (**2**):* $[\alpha]^{26}_D$ +28.0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 223 (3.80) nm; IR (neat) ν_{max} 3422, 2923, 1635, 1460, 1388, 1107, 1055, 1020 cm^{-1}; ^{1}H NMR (CD_3OD) see Table 1; ^{13}C NMR (CD_3OD) see Table 3; ESIMS *m/z* 361 $[M + Na]^+$; HRESIMS *m/z* 361.2359 $[M + Na]^+$ (calcd for $C_{20}H_{34}O_4Na$, 361.2349).

*Reaction of **2** with (R)-MTPA.*[14,15] (*R*)-MTPA (25 mg) and thionyl chloride (1 mL) were mixed and heated under reflux for 3 h. Then, excess thionyl chloride was removed, a solution of **2** (8 mg) in 4 mL of dry CH_2Cl_2, triethylamine (0.1 mL), and (dimethylamino)pyridine (10 mg) were added, and the solution was stirred at room temperature for 16 h. The reaction mixture was purified by preparative TLC (1% MeOH/$CHCl_3$) to yield the (*R*)-MTPA ester **2-RE**: ^{1}H NMR ($CDCl_3$, 600 MHz) δ 1.18 (3H, s, H_3-17), 5.00 (1H, d, *J* = 9.6 Hz, H-12), 5.08 (1H, d, *J* = 11.4 Hz, H-15a), 5.32 (1H, d, *J* = 18.0 Hz, H-15b), 5.13 (1H, s, H-16a), 5.28 (1H, s, H-16b), 6.32 (1H, dd, *J* = 10.8, 18.0 Hz, H-14).

*Reaction of **2** with (S)-MTPA.*[14,15] (*S*)-MTPA (25 mg) and thionyl chloride (1 mL) were mixed and heated under reflux for 3 h. Then, excess thionyl chloride was removed, a solution of **2** (8 mg) in 4 mL of dry CH_2Cl_2, triethylamine (0.1 mL), and (dimethylamino)pyridine (10 mg) were added, and the solution was stirred at room temperature for 16 h. The reaction mixture was purified by preparative TLC (1% MeOH/$CHCl_3$) to yield the (*S*)-MTPA ester **2-SE**: ^{1}H NMR ($CDCl_3$, 600 MHz) δ 1.16 (3H, s, H_3-17), 5.04 (1H, d, *J* = 9.6 Hz, H-12), 5.11 (1H, d, *J* = 12.0 Hz, H-15a), 5.33 (1H, d, *J*

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= 18.0 Hz, H-15b), 5.15 (1H, s, H-16a), 5.30 (1H, s, H-16b), 6.33 (1H, dd, J = 11.4, 17.4 Hz, H-14).

*(12R)-Epiblumdane (**3**):* $[\alpha]^{26}_{D}$ +47.2 (c 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 223 (3.44) nm; IR (neat) ν_{max} 3417, 2923, 1601, 1458, 1391, 1250, 1102 cm^{-1}; ^1H NMR (CD_3OD) see Table 1; ^{13}C NMR (CD_3OD) see Table 3; ESIMS m/z 361 $[M + Na]^+$; HRESIMS m/z 361.2354 $[M + Na]^+$ (calcd for $C_{20}H_{34}O_4Na$, 361.2349).

*6α-O-(3-Methyl-2-butenoyl)sterebin A (**5**):* $[\alpha]^{26}_{D}$ +42.0 (c 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 221 (4.12) nm; IR (neat) ν_{max} 3444, 2930, 1719, 1648, 1388, 1233, 1151, 1077 cm^{-1}; ^1H NMR (CD_3OD) see Table 2; ^{13}C NMR (CD_3OD) see Table 3; ESIMS m/z 415 $[M + Na]^+$; HRESIMS m/z 415.2469 $[M + Na]^+$ (calcd for $C_{23}H_{36}O_5Na$, 415.2455).

*6α-O-Angeloylsterebin A (**6**):* $[\alpha]^{26}_{D}$ +68.8 (c 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 223 (4.26) nm; IR (neat) ν_{max} 3439, 2930, 1712, 1672, 1460, 1388, 1371, 1262, 1235, 1153, 1084, 1047 cm^{-1}; ^1H NMR (CD_3OD) see Table 2; ^{13}C NMR (CD_3OD) see Table 3; HRESIMS m/z 415.2471 $[M + Na]^+$ (calcd for $C_{23}H_{36}O_5Na$, 415.2460).

*6α-O-Isovalerylsterebin A (**7**):* $[\alpha]^{26}_{D}$ +76.2 (c 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 226 (4.10) nm; IR (neat) ν_{max} 3434, 2957, 2928, 1722, 1675, 1625, 1470, 1371, 1297, 1262, 1193, 1124, 1082, 1049, 1000 cm^{-1}; ^1H NMR (CD_3OD) see Table 2; ^{13}C NMR (CD_3OD) see Table 3; ESIMS m/z 417 $[M + Na]^+$; HRESIMS m/z 417.2627 $[M + Na]^+$ (calcd for $C_{23}H_{38}O_5Na$, 417.2617).

Mice. Female BALB/c mice age 5−6 weeks were purchased from the National Laboratory Animal Breeding and Research Center in Taiwan. All animal studies and the experimental procedures were approved by the Institutional Animal Care and Use Committee of the National Research Institute of Chinese Medicine (IACUC106-355-1).

Isolation of gMDSCs. Mouse mammary carcinoma 4T1 cells were inoculated into the mammary fat-pad of test mice for 20 days, as described previously.[17] To isolate granulocytic MDSCs, splenocytes of 4T1 tumor-bearing mice were collected. Ly-6G+ cells were isolated from the splenocytes with the Anti-Ly-6G MicroBead kit, following the manufacturer's instructions (Miltenyi Biotec).

Measurement of Cytokines. Altogether 5×10^5 RAW 264.7 murine macrophages were cultured in 12-well culture plates for 24 h. The cells were treated with or without the test compounds (20 or 40 μM) in the presence or absence of LPS (1 μg/mL). The production of TNF-α in the cell cultures was determined by using commercial ELISA kits (R&D Systems).

Flow Cytometry. Altogether 5×10^5 gMDSCs were cultured in 12-well culture plates with or without the test compounds (20 or 40 μM) in the presence or absence of LPS (1 μg/mL) for 24 h. The cells were harvested and washed twice with phosphate-buffered saline. The PD-L1 expression level of gMDSCs was determined by flow cytometry, as described previously.[18] In brief, the gMDSCs were stained with APC-conjugated PD-L1 monoclonal antibodies for 20 min at 4 °C and then fixed in 1% paraformaldehyde. Cell analysis was performed by using a flow cytometer (BD Pharmingen).

Statistical Analysis. Data are presented as the mean ± SD. Statistical analyses were performed with GraphPad Prism software. A one-way ANOVA analysis was used to compare the multiple data sets.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.9b00674.

1D and 2D NMR spectra for compounds **1**−**8** (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*Tel: +886-2-28201999, ext. 7101. Fax: +886-2-2826 4276. E-mail: lclin@nricm.edu.tw.

ORCID

Lie-Chwen Lin: 0000-0002-6426-9696

Notes

The authors declare no competing financial interest.

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